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Upf1p, a highly conserved protein required for nonsense-mediated mRNA decay, interacts with the nuclear pore proteins Nup100p and Nup116p

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Abstract

Saccharomyces cerevisiae Upf1p is a 971-amino-acid protein that is required for the nonsense-mediated mRNA decay (NMD) pathway, a pathway that degrades mRNAs with premature translational termination codons. We have identified a two-hybrid interaction between Upf1p and the nuclear pore (Nup) proteins, Nup100p and Nup116p. Both nucleoporins predominantly localize to the cytoplasmic side of the nuclear pore and participate in mRNA transport. The two-hybrid interaction between Upf1p and the nuclear pore proteins, Nup100p and Nup116p, is dependent on the presence of the C-terminal 158 amino acids of Upf1p. Nup100p and Nup116p can be co-immunoprecipitated from whole-cell extracts with Upf1p, confirming in vitro the interaction identified by the two-hybrid analysis. Finally, we see a genetic interaction between *UPF1* and *NUP100*. The growth of *upf1Δ, can1-100* cells is inhibited by canavanine. The deletion of *NUP100* allows *upf1Δ, can1-100* cells to grow in the presence of canavanine. Physiologically, the interaction between Upf1p and the nuclear pore proteins, Nup100p and Nup116p, is significant because it suggests a mechanism to ensure that Upf1p associates with newly synthesized mRNA as it is transported from the nucleus to the cytoplasm prior to the pioneer round of translation.

Keywords: mRNA surveillance; mRNA transport

1. Introduction

mRNAs containing premature translation termination codons (nonsense mRNAs) are degraded faster than their wild-type counterparts by nonsense-mediated mRNA decay (NMD; reviewed in Culbertson and Leeds, 2003). Nonsense mRNAs can arise by the transcription of genes with nonsense or frame-shift mutations and from errors in processing or transcription. The rapid decay of nonsense mRNA prevents the synthesis of potentially harmful truncated peptides. Thus, NMD ensures that only properly processed mRNAs encoding functional polypeptides persist.

NMD has been intensively studied in the yeast *Saccharomyces cerevisiae*, *Caenorabditis elegans*, and vertebrates (reviewed in Culbertson and Leeds, 2003).

Abbreviations: NMD, nonsense-mediated mRNA decay; Upf, up frameshift; Nup, nuclear pore; eRF, eukaryotic release factor; DSE, downstream sequence element; PCR, polymerase chain reaction; YEPD/YPD, yeast extract, peptone, and dextrose medium; YAPD, YPD supplemented with adenine; 3-AT, 3-aminotriazole; EDTA, ethylenediaminetetraacetic acid; NaCl, sodium chloride; Tris, tris(hydroxymethyl)aminomethane; PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HA, influenza hemagglutininin epitope; GLFG, motif consisting of repeats of glycine, leucine, phenylalanine, and glycine interspersed with glutamine-, asparagine-, serine-, and threonine-rich spacers; DNA, deoxyribonucleic acid; tRNA, transfer ribonucleic acid; rRNA, ribosomal ribonucleic acid; NRM, nucleoporin RNA-binding motif; GLEBS, Gle2p-binding sequence; $GAL4_{BD}$, Gal4 binding domain; $GAL4_{AD}$, Gal4 activation domain; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel elecrophoresis.

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Yeast has three NMD-specific proteins: Upf1p, Upf2p, and Upf3p (Leeds et al., 1992; Cui et al., 1995; Lee and Culbertson, 1995). Sixteen *UPF1*, 11 *UPF2*, and 7 *UPF3* orthologues have been found in a diverse array of eukaryotes (Culbertson and Leeds, 2003). These proteins act in a single pathway and, more recently, are proposed to be part of a dynamic surveillance complex that acts as a sensor for nonsense mRNAs (Atkin et al., 1997; Lykke-Andersen et al., 2001).

Upf1p is critical for NMD. It aids in the termination of translation at premature translation termination codon, by interacting with the eukaryotic translation release factors eRF1 and eRF3 (Czaplinski et al., 1998). Then, it triggers decapping by interacting with the decapping complex (Dunckley and Parker, 1999). Decapped mRNAs are then rapidly degraded by Xrn1p, a 5′→3′ exoribonuclease.

In the current view of the NMD pathway, premature termination codons are recognized through cooperative interactions between the Upf proteins and a protein complex, which is deposited either immediately upstream of exon–exon junctions on mammalian mRNAs or on the downstream sequence element (DSE) of yeast mRNAs (Gonzalez et al., 2000; Le Hir et al., 2001). First, Upf3p and, then, Upf2p join the complex (Lykke-Andersen et al., 2001). It has been proposed that Upf1p is recruited to the complex when translation terminates upstream of the complex, triggering NMD thorough interactions with Upf2p and the translation release factors (Czaplinski et al., 1998; Le Hir et al., 2001). On the other hand, if translation terminates downstream of the mark, Upf1p is not recruited to the complex and the mRNA escapes NMD.

mRNA degradation via NMD requires at least one round of translation, and it might even occur during the pioneer round of translation (Ishigaki et al., 2001). CBP80 and CBP20 form a complex that binds to the mRNA cap while it is still in the nucleus. The complex travels with the mRNA to the cytoplasm. CBP80/CBP20-bound mRNA is then translated at least once before eIF-4E replaces the CBP80/CBP20 complex. eIF-4E functions in subsequent translation initiation. Three lines of evidence suggest that NMD takes place on CBP80-bound mRNA: (1) CBP80- and eIF4E-bound nonsense mRNAs are similarly reduced in abundance (Ishigaki et al., 2001). (2) Consistent with the dependence of NMD on translation, a cognate suppressor transfer ribonucleic acid (tRNA) or inhibition of translation by cycloheximide stabilized the CBP-bound nonsense mRNA (Ishigaki et al., 2001). (3) Finally, hUpf2p, hUpf3p, and the components of the exon–exon junction complex copurify with CBP80-bound but not eIF-4E-bound mRNA (Ishigaki et al., 2001).

In this study, we observe an interaction between yeast Upf1p and two components of the nuclear pore (Nup) complex, Nup100p and Nup116p. Both of these nucleoporins predominantly localize to the cytoplasmic side of the nuclear pore and participate in mRNA transport (reviewed in Suntharalingam and Wente, 2003). The observation of an interaction between Upf1p and these nuclear pore proteins suggests that Upf1p may associate with newly synthesized mRNA as it is transported from the nucleus to the cytoplasm prior to the pioneer round of translation and, thus, earlier than previously proposed.

2. Materials and methods

2.1. Yeast strains, genetic methods, and culture conditions

The *S. cerevisiae* strains are described in Table 1. The yeast strains were constructed, grown, and maintained by standard procedures (Ausubel et al., 1998). SSX-leu-his plates were prepared as in Chien et al. (1991). Yeast were transformed using the lithium acetate method (Ausubel et al., 1998).

2.2. Bacterial strains and plasmid DNA construction

Escherichia coli DH5α (Life Technologies, Rockville, MD) was used for the preparation of plasmid deoxyribonucleic acid (DNA). The methods used for the growth, maintenance, and transformation of *E. coli* are in Ausubel et al. (1998). Plasmid DNAs were prepared from *E. coli* using a QIAprep spin plasmid miniprep kit (Qiagen, Chatsworth, CA). New plasmids were constructed using standard methods (Ausubel et al., 1998). The plasmid DNAs are listed in Table 1.

pAA158 (Table 1) was used to screen yeast libraries for proteins that interact with Upf1p. A DNA fragment coding for the region of Upf1p with sequence similarity to Sen1p was amplified by polymerase chain reaction (PCR) using oligonucleotides oAA16 (5′-dCGA TGC GTC CGG CGT AGA GGA) and oAA18 (5′-dGGC GAA TTC TCT GCT TCT GAT ACA AAA CG). oAA18 was designed to create an *Eco*RI site (underlined) at nucleotides +1569 to +1574 within the *UPF1* ORF. The DNA fragment was amplified from pPL46 (Leeds et al., 1992) using *Taq* DNA polymerase (Cetus, Emerywille, CA). pPL46 contains a 4.0-kb *Eco*RI-*Bam*HI fragment of DNA carrying the *UPF1* gene cloned into the *Eco*R1-*Bam*HI sites of YIp5. The PCR product was digested with *Eco*RI and *Bam*HI, cloned into the *Eco*R1-*Bam*HI sites of pUC19 and sequenced using the Sequenase 2.0 kit (United States Biochemical, Cleveland, OH). An *Eco*RI-*Bam*HI fragment containing wild-type *UPF1* sequence was subcloned into the *Eco*R-*Bam*HI sites of pMA424 (Chien et al., 1991), creating pAA158. pAA158 contains an in-frame fusion of the *GAL4* DNA binding domain and the 3′ half of *UPF1* under the control of the constitutive *ADH1* promoter. On its own, this plasmid did not activate the expression of the *lacZ* reporter gene in GGY1::171 (data not shown).

pAA303 contains an in-frame fusion of the *GAL4* DNA-binding domain and the 3′ half of *UPF1*. It was constructed by subcloning an *Eco*RI-*Bam*HI fragment from pAA158